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Final Report: A CRISPR-based MLST Scheme for Understanding the Population Biology and Epidemiology of Salmonella Enterica

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14. ABSTRACT

Salmonella enterica subsp. enterica is the most common cause of bacterial foodborne illness in the United States. With Army ARO funds, we have investigated over the past three years whether polymorphic regions called CRISPR can used to differentiate strains of this pathogen for outbreak investigations. We have also been investigating whether CRISPR in Salmonella protects the bacteria against foreign DNA as described in other systems, or whether it has alternative functions. Here, we report that CRISPR can be used to subtype Salmonella enterica serovariants Heidelberg and Typhimurium, and discuss findings from sequence analysis of over 600 strains

15. SUBJECT TERMS

CRISPR, Salmonella, molecular subtyping, bacterial genetics

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Report Title

Final Report: A CRISPR-based MLST Scheme for Understanding the Population Biology and Epidemiology of Salmonella Enterica

ABSTRACT

Salmonella enterica subsp. enterica is the most common cause of bacterial foodborne illness in the United States. With Army ARO funds, we have investigated over the past three years whether polymorphic regions called CRISPR can used to differentiate strains of this pathogen for outbreak investigations. We have also been investigating whether CRISPR in Salmonella protects the bacteria against foreign DNA as described in other systems, or whether it has alternative functions. Here, we report that CRISPR can be used to subtype Salmonella enterica serovariants Heidelberg and Typhimurium, and discuss findings from sequence analysis of over 600 strains performed over the course of this study.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
05/22/2015	5.00	N. Shariat, R. E. Timme, J. B. Pettengill, R. Barrangou, E. G. Dudley. Characterization and evolution of Salmonella CRISPR-Cas systems, Microbiology, (12 2014): 0. doi: 10.1099/mic.0.000005
05/22/2015	8.00	N. Shariat, E. G. Dudley. CRISPRs: Molecular Signatures Used for Pathogen Subtyping, Applied and Environmental Microbiology, (10 2013): 0. doi: 10.1128/AEM.02790-13
05/22/2015	6.00	Xiangyu Deng, Nikki Shariat, Elizabeth M. Driebe, Chandler C. Roe, Beth Tolar, Eija Trees, Paul Keim, Wei Zhang, Edward G. Dudley, Patricia I. Fields, David M. Engelthaler, N. A. Ledeboer. Comparative Analysis of Subtyping Methods against a Whole-Genome-Sequencing Standard for Salmonella enterica Serotype Enteritidis, Journal of Clinical Microbiology, (01 2015): 0. doi: 10.1128/JCM.02332-14
05/26/2015	9.00	Nikki Shariat, Carol H Sandt, Michael J DiMarzio, Rodolphe Barrangou, Edward G Dudley. CRISPR-MVLST subtyping of Salmonella enterica subsp. enterica serovars Typhimurium and Heidelberg and application in identifying outbreak isolates, BMC Microbiology, (11 2013): 254. doi: 10.1186/1471-2180-13-254
05/26/2015	16.00	Edward G Dudley, Nikki Shariat. Where are we heading with Salmonella molecular subtyping, Future Microbiology, (10 2013): 1231. doi: 10.2217/fmb.13.107
05/28/2013	2.00	Nikki Shariat, Michael J. DiMarzio, Shuang Yin, Lisa Dettinger, Carol H. Sandt, James R. Lute, Rodolphe Barrangou, Edward G. Dudley. The combination of CRISPR-MVLST and PFGE provides increased discriminatory power for differentiating human clinical isolates of Salmonella enterica subsp. enterica serovar Enteritidis, Food Microbiology, (05 2013): 164. doi: 10.1016/j.fm.2012.11.012
08/23/2013	3.00	M. DiMarzio, N. Shariat, S. Kariyawasam, R. Barrangou, E. G. Dudley. Antibiotic Resistance in Salmonella enterica Serovar Typhimurium Associates with CRISPR Sequence Type, Antimicrobial Agents and Chemotherapy, (06 2013): 4282. doi: 10.1128/AAC.00913-13
08/23/2013	4.00	N. Shariat, M. K. Kirchner, C. H. Sandt, E. Trees, R. Barrangou, E. G. Dudley. Subtyping of Salmonella enterica Serovar Newport Outbreak Isolates by CRISPR-MVLST and Determination of the Relationship between CRISPR-MVLST and PFGE Results, Journal of Clinical Microbiology, (05 2013): 2328. doi: 10.1128/JCM.00608-13
TOTAL:		**8**

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper
05/26/2015	12.00 Nikki Shariat, Carol H. Sandt, Rodolphe Barrangou, Edward G Dudley. CRISPR analysis of clinical Salmonella isolates, CRISPR Evolution, Mechanisms, and Infection. 17-JUN-13, . : ,
05/26/2015	13.00 Nikki Shariat, Ruth Timme, James Pettengill, Rodolphe Barrangou, Edward G. Dudley. Characterization of CRISPR-Cas in Salmonella, 3rd European CRISPR meeting. 14-MAY-14, . : ,
05/26/2015	14.00 Margaret K. Kirchner, Nikki Shariat, Katherine Very, Bhushan M. Jayarao, Edward G. Dudley. Raccoons as a reservoir for Salmonella, 2014 Allegheny Branch of the American Society for Microbiology. 07-NOV-14, . : ,
05/26/2015	15.00 Michael DiMarzio, Nikki Shariat, Subhashinie Kariyawasam, Rodolphe Barrangou, Edward Dudley. CRISPR-MVLST identifies populations of Salmonella Typhimurium with differences in distribution and antibiotic resistance, 2013 International Association of Food Protection. 28-JUL-13, . : ,

TOTAL: **4**

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper
05/26/2015 11.00	Nikki Shariat, Edward G Dudley. Where are we heading with Salmonella molecular subtyping, Future Microbiology (10 2013)

TOTAL: **1**

Number of Manuscripts:

Books

Received	Book

TOTAL:

Received	Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Ms. Margaret Kirchner, undergraduate student working on this project, won first place in the undergraduate molecular microbiology poster competition at the 2014 Allegheny Branch of the American Society for Microbiology meeting (Lycoming College)

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Nikki Shariat	1.00
FTE Equivalent:	**1.00**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Edward G Dudley	0.05	
Stephen J. Knabel	0.05	
FTE Equivalent:	**0.10**	
Total Number:	**2**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Margaret Kirchner	0.00	Food Science
FTE Equivalent:	**0.00**	
Total Number:	**1**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	1.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:	1.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:	1.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	1.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	1.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME PERCENT_SUPPORTED

FTE Equivalent:

Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Research update:

CRISPR-MVLST

Since our last report (August 2013), we completed CRISPR-MVLST analysis of two more prevalent Salmonella serovars: Typhimurium and Heidelberg. Collectively, these serovars account for a fifth of all annual salmonellosis cases in the United States. We analyzed a broad set of 175 S. Heidelberg and S. Typhimurium isolates collected over a five-year period by the Pennsylvania Department of Health. We identified 21 Heidelberg Sequence Types (HSTs) and 37 Typhimurium STs (TSTs) that were represented by 27 and 45 PFGE pulsotypes, respectively, and determined the discriminatory ability of each method.

For S. Heidelberg, our data shows that combined typing by both CRISPR-MVLST and PFGE provided a discriminatory power of 0.9213. Importantly, CRISPR-MVLST was able to separate common PFGE patterns such as JF6X01.0022 into distinct STs, thus providing significantly greater discriminatory power. Conversely, we show that subtyping by either CRISPR-MVLST or PFGE independently provides a sufficient discriminatory power (0.9345 and 0.9456, respectively) for S. Typhimurium. Additionally, using isolates from two S. Typhimurium outbreaks, we demonstrate that CRISPR-MVLST provides excellent epidemiologic concordance.

Cumulatively, with funds from ARO, we have shown that CRISPR-MVLST is a comparable method to PFGE, the current 'gold standard' in Salmonella subtyping. Given the rapidity and tractability of CRISPR-MVLST, we extended our studies to analyze Salmonella isolates from outbreaks (S. Newport and S. Typhimurium). We also participated in a collaboration initiated by the Food and Drug Administration that involves twelve different methods of Salmonella typing analysis (see manuscripts in preparation). In this study, CRISPR-MVLST did extremely well, producing subtyping results that were discriminatory and epidemiologically concordant as well as being the least expensive method employed.

In addition, Drs. Dudley and Shariat also wrote a review, "CRISPRs: Molecular Signatures used for Pathogen Subtyping" for Applied and Environmental Microbiology, which is the first review article to be published on CRISPR-based typing methodology and is expected to be highly cited in the future.

Salmonella CRISPRs

Beyond subtyping approaches, we were motivated to understand the biology of CRISPR-Cas systems in Salmonella. We performed in-depth sequence analysis of the CRISPR-Cas systems in >600 Salmonella, representing four clinically prevalent serovars. Each CRISPR-Cas feature (cas genes, leader sequences and array) is extremely conserved in Salmonella, and the CRISPR1 locus is more highly conserved than CRISPR2. Array composition is serovar-specific, though no convincing evidence of recent spacer acquisition against exogenous nucleic acids exists.

Our observations reflect historical CRISPR-Cas immune activity, showing that this locus has ceased undergoing adaptive events. Intriguingly, the high level of conservation across divergent serovars shows that the genetic integrity of these inactive loci is maintained over time, contrasting with the canonical view that inactive CRISPR loci degenerate over time. This thorough characterization of Salmonella CRISPR-Cas systems presents new insights into Salmonella CRISPR evolution, particularly with respect to cas gene conservation, leader sequences, organization of direct repeats and protospacer matches. Collectively, our data suggests that Salmonella CRISPR-Cas systems are no longer immunogenic; rather their impressive conservation indicates they may have an alternative function in Salmonella.

Overall impact

Over the duration of this grant, we conclusively demonstrated that CRISPR can be developed into an effective subtyping marker for tracking foodborne outbreaks of Salmonella enterica serovars Enteritidis, Typhimurium, Heidelberg, and Newport. We worked with several government agencies including the Food and Drug Administration, Centers for Disease Control, and Pennsylvania Department of Health to develop this method using patient isolates. Drs. Dudley and Shariat published seven peer-reviewed articles (2 more in preparation or submitted), wrote two invited editorials, were authors on five posters presented at scientific meetings, and presented this work 15 times at scientific meetings and academic institutions. A comprehensive list of these is at the end of this report.

Future plans

Dr. Shariat moved to Gettysburg College in June 2015 to begin a tenure-track position as an Assistant Professor in the Department of Biology. In her research lab, her focus will continue to be on Salmonella CRISPR – discerning their function and evolution and also their utility as a subtyping tool.

Publications:

Shariat N, Timme R, Pettengill J, Barrangou R and Dudley EG. 2015. Characterization and Evolution of Salmonella CRISPR-Cas systems. Microbiology. 161:374-386.

Deng X, Shariat N, Driebe EM, Tolar B, Trees E, Keim P, Zhang W, Dudley EG, Fields PI, and David M. Engelthaler. 2015. Whole genome sequencing-based benchmarking of subtyping methods for Salmonella enterica serotype Enteritidis. Journal of

Clinical Microbiology. 53:212-218.

Shariat N and Dudley EG. 2014. CRISPRs: Molecular Signatures used for Pathogen Subtyping. Applied and Environmental Microbiology. 80:430-439.

Shariat N, Sandt CH, DiMarzio MJ, Barrangou R and Dudley EG. 2013. CRISPR-MVLST subtyping of Salmonella enterica subsp. enterica serovars Typhimurium and Heidelberg and application in identifying outbreak isolates. BMC Microbiology. 13: 254

DiMarzio MJ, Shariat N, Kariyawasam S and Dudley EG. 2013. CRISPR-MVLST identifies populations of S. Typhimurium with differences in distribution and antibiotic resistance. Antimicrobial Agents and Chemotherapy. 57:4282-4289.

Shariat N, Kirchner MA, Sandt CH, Barrangou R and Dudley EG. 2013.
Application of CRISPR-MVLST to distinguish Salmonella Newport outbreak isolates in the United States. Journal of Clinical Microbiology. 51:2328-36.

Shariat N, DiMarzio MJ, Yin S, Dettinger L, Sandt CH, Lute JR, Barrangou R and Dudley EG. 2013. The combination of CRISPR-MVLST and PFGE provides increased discriminatory power for differentiating human clinical isolates of Salmonella enterica subsp. enterica serovar Enteritidis. Food Microbiology. 34:164-173.

Invited editorials:

Dudley, E. G., M. DiMarzio, and N. Shariat. 2014. CRISPRs: molecular markers for tracking antibiotic resistant strains of Salmonella enterica. Published in the Winter 2014 newsletter of the Alliance for the Prudent Use of Antibiotics (http://www.tufts.edu/med/apua/news/newsletter_64_563530622.pdf).

Shariat N and Dudley EG. 2013. Where are we heading with Salmonella molecular subtyping? Future Microbiology. 8:1231-1233.

Manuscripts submitted and in preparation:

Very K, Kirchner M, Shariat N, Cottrell W, Sandt CH, Dudley EG, and Jayarao BM.
Prevalence and spatial distribution of Salmonella enterica infections in the Pennsylvania common raccoon (Procyon Lotor) (submitted)

Andrzejewski D, Brown E, Burrows E, Czajka J, Driebe E, Dudley E, Elkins C, Engelthaler D, Evans P, Gorham S, Hanes D, Keys C, Jackson S, McFarland M, Musser S, Ottesen A, Patel I, Pettengill J, Shariat N, Soler-Garcia A, Strain E, Timme R Validation of Molecular Tools for Foodborne Outbreak Investigation (in preparation).

Oral presentations:

Shariat, N. Juniata College, Department of Biology, 2015
“CRISPR-Cas Systems: Bacterial adaptive immunity and its applications”

Shariat, N. Mount Aloysius College, Biology 2015
“CRISPRs: Immunity and Applications”

Dudley, E.G. CRISPR typing of Escherichia coli and Salmonella enterica 2015
E. coli Workshop, Penn State, University Park, PA.

Kirchner, M. K., N. Shariat, K. Very, B. M. Jayarao, and E. G. Dudley. 2014. Raccoons as a reservoir for Salmonella? Presented at the Allegheny Branch of the American Society for Microbiology Meeting, Lycoming College, PA. Lead author M.K.K won 1st place in the undergraduate molecular microbiology poster competition.

Shariat, N. Allegheny Branch – American Society of Microbiology, Annual Meeting, Lycoming College, Penn. 2014
“An Evolutionary Perspective of Salmonella CRISPR-Cas systems”

Shariat, N. University of Memphis, Department of Biology, Seminar Series 2014
“Do CRISPRs Provide Immunity? Characterization of CRISPR-Cas in Salmonella”

Shariat, N. Oberlin University, Biology Department, Seminar 2014

“CRISPR-Cas Systems in Salmonella – Do They Provide Immunity?”

Shariat, N. Juniata College, Department of Biology, Seminar Series 2014
“Do CRISPRs Provide Immunity? Characterization of CRISPR-Cas in Salmonella”

Shariat, N. University of Southern Mississippi, Department of Biological Sciences Seminar 2014. “Evolution and Function of Salmonella CRISPR-Cas Systems”

Shariat, N. CRISPR Workshop, Penn State University 2013. “CRISPR-cas Systems: An overview of bacterial adaptive immunity and its applications”

Dudley, E. G. CRISPR in Salmonella. Presented to the National Turkey Federation Technical and Regulatory Committee, Washington, DC. October 1, 2013.

Dudley, E. G. CRISPR: a new target for molecular tracking of Escherichia coli and other pathogens. Grocery Manufacturer’s Association 2013 Scientific Forum (Washington, DC). April 1, 2013.

Shariat, N. 7th Annual Workshop in virus Evolution, State College, Pennsylvania 2013 “Evolutionary Analysis of CRISPR elements in clinical isolates of Salmonella enterica”

Shariat, N. Center for Food Manufacturing Meeting, State College, Pennsylvania 2012. “Determining the Functionality of CRISPR-MVLST as a Subtyping Method in Salmonella”

Shariat, N. 4th Annual CRISPR Meeting, International Conference, Berkeley, California 2012. “CRISPR Subtyping of Salmonella enterica”

Poster presentations:
Deng, X., N. Shariat, E. Driebe, B. Tolar, E. Trees, W. Zhang, E. G. Dudley, P. Fields, and D. Engelthaler. 2014. Whole genome sequencing based benchmarking of subtyping methods for Salmonella enterica serotype Enteritidis. Presented at the 2014 International Association for Food Protection Meeting, Indianapolis, IN.

Shariat, N., Timme, R., Pettengill, J., Barrangou, R. and Dudley, E.G. 6th Annual CRISPR Meeting, International Conference, Berlin, Germany 2014
“Characterization of CRISPR-Cas in Salmonella”

Shariat, N, Sandt, C.H., Barrangou, R. and Dudley, E.G. 5th Annual CRISPR Meeting, International Conference, St. Andrews, United Kingdom 2013
“CRISPR analysis of clinical Salmonella isolates”

DiMarzio, M., N. Shariat, S. Kariyawasam, R. Barrangou, and E. G. Dudley. 2013. CRISPR-MVLST identifies populations of Salmonella Typhimurium with differences in distribution and antibiotic resistance. Presented at the 2013 International Association for Food Protection Meeting, Charlotte, North Carolina.

Shariat, N. DiMarzio, MJ, Yin, S, Barrangou, R and Dudley, E.G. American Society of Microbiology - Annual Meeting, International Conference, San Francisco, California 2012. “CRISPR Subtyping of an Unbiased Set of Clinical Salmonella Enteritidis Isolates” Poster

Technology Transfer